

WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a nucleotide sequence encoding a chimeric protein, said chimeric protein comprises, from N-terminus to
5 C-terminus:

- a) a first peptidyl fragment consisting of an amino acid sequence that has at least 40% identity to residues 1-50 of the B chain of native monellin, in which the percentage identity is determined over an amino acid sequence of identical size to the B chain of native
10 monellin;
- b) a peptidyl bond, or a second peptidyl fragment consisting of 1-12 amino acids; and
- c) a third peptidyl fragment consisting of an amino acid sequence that has at least 40% identity to residues 1-45 of the A chain of native
15 monellin, in which the percentage identity is determined over an amino acid sequence of identical size to the A chain of native monellin,

wherein said chimeric protein is stable and a given amount of said chimeric protein is at least 100-fold sweet as compared to the identical amount of sucrose, and
20 within said nucleic acid, codons which are preferably used by yeast cells are used.

2. The isolated nucleic acid of claim 1, wherein the first peptidyl fragment consists of an amino acid sequence that has at least 60% identity to the B chain of native monellin.

3. The isolated nucleic acid of claim 1, wherein the first
25 peptidyl fragment consists of an amino acid sequence that has at least 90% identity to the B chain of native monellin.

4. The isolated nucleic acid of claim 1, wherein the first peptidyl fragment consists of the amino acid residues 1-50 of the B chain of native monellin depicted as the amino acid residues 1-50 in Figure 1 (SEQ ID NO:5).

30 5. The isolated nucleic acid of claim 1, wherein the second peptidyl fragment consists of the amino acid residue Gly.

6. The isolated nucleic acid of claim 1, wherein the second peptidyl fragment consists of the amino acid sequence Gly-Gly-Gly-Ser (SEQ ID-NO:2).
7. The isolated nucleic acid of claim 1, wherein the second
5 peptidyl fragment consists of the amino acid sequence Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser-Gly-Gly-Gly-Ser (SEQ ID NO:1).
8. The isolated nucleic acid of claim 1, wherein the third peptidyl fragment consists of an amino acid sequence that has at least 60% identity to the A chain of native monellin.
- 10 9. The isolated nucleic acid of claim 1, wherein the third peptidyl fragment consists of an amino acid sequence that has at least 90% identity to the A chain of native monellin.
10. The isolated nucleic acid of claim 1, wherein the third peptidyl fragment consists of the amino acid residues 1-45 of the A chain of native
15 monellin depicted as the amino acid residues 52-96 in Figure 1 (SEQ ID NO:5).
11. The isolated nucleic acid of claim 1 which nucleic acid encodes the amino acid residues 1-96 of Figure 1 (SEQ ID NO:5).
12. The isolated nucleic acid of claim 1, wherein the chimeric protein is capable of being immunoreactively bound by an anti-monellin antibody.
- 20 13. The isolated nucleic acid of claim 1, wherein the chimeric protein is capable of being immunoreactively bound by an anti-thaumatococcus antibody.
14. The isolated nucleic acid of claim 1, wherein the chimeric protein further comprises an amino acid sequence which is capable of directing secretion of said chimeric protein from *Pichia pastoris*.
- 25 15. The isolated nucleic acid of claim 14, wherein the secretion-directing sequence is an endogenous signal sequence of *Pichia pastoris*.
16. The isolated nucleic acid of claim 15, wherein the endogenous signal sequence is selected from the group consisting of the signal sequence of *Pichia pastoris* acid phosphatase, *Pichia pastoris* aspartic proteinase
30 and *Pichia pastoris* carboxypeptidase Y encoded by *Pichia pastoris* PRC1.

17. The isolated nucleic acid of claim 14, wherein the secretion-directing sequence is a yeast signal sequence, wherein said yeast is not *Pichia pastoris*.

18. The isolated nucleic acid of claim 17, wherein the yeast
5 signal sequence is a signal sequence from *Saccharomyces cerevisiae*.

19. The isolated nucleic acid of claim 18, wherein the *Saccharomyces cerevisiae* signal sequence is selected from the group consisting of the signal sequence of *Saccharomyces cerevisiae* SUC 2 and *Saccharomyces cerevisiae* mating pheromone α -factor.

20. The isolated nucleic acid of claim 19, wherein the
10 *Saccharomyces cerevisiae* signal sequence is the signal sequence of *Saccharomyces cerevisiae* mating pheromone α -factor.

21. The isolated nucleic acid of claim 11, further comprising an amino acid sequence which is capable of directing secretion of said chimeric
15 protein from *Pichia pastoris*.

22. The isolated nucleic acid of claim 21, wherein the secretion-directing sequence is the signal sequence of *Saccharomyces cerevisiae* mating pheromone α -factor.

23. The isolated nucleic acid of claim 14, wherein the secretion-
20 directing sequence is selected from the group consisting of the signal sequence of *Aspergillus giganteus* alpha-Sarcin, alpha-N-Acetylgalactosaminidase, OmpA protein, the mouse alpha-factor (cCell), the pepper endo-beta-1,4-glucanases, the laccase isolated from the ligninolytic fungus *Trametes*, murine lysosomal acid alpha-mannosidase, the porcine inhibitor of carbonic anhydrase, *Aspergillus*
25 *awamori* glucoamylase, mouse major urinary protein, pho1, rabbit angiotensin-converting enzyme (ACE), and the bacterial thermostable alpha amylase.

24. The nucleic acid of claim 1, wherein said nucleic acid is a DNA.

25. An isolated nucleic acid comprising a nucleotide sequence
30 complementary to the nucleotide sequence of claim 1.

24

26. An isolated nucleic acid hybridizable to the DNA sequence of claim 24.

27. The DNA of claim 24, further comprising a promoter which is capable of directing protein expression in *Pichia pastoris*.

5 28. The DNA of claim 27, wherein the promoter is an endogenous promoter of *Pichia pastoris*.

29. The DNA of claim 28, wherein the endogenous promoter is the promoter of *Pichia pastoris* glyceraldehyde-3-phosphate dehydrogenase.

10 30. The DNA of claim 24 said DNA encodes the amino acid residues 1-96 of Figure 1 (SEQ ID NO:5) and said DNA further comprises the promoter of *Pichia pastoris* glyceraldehyde-3-phosphate dehydrogenase and the signal sequence of *Saccharomyces cerevisiae* mating pheromone α -factor.

31. The DNA of claim 24, wherein the codons which are preferably used by *Pichia pastoris* cells are used.

15 32. A DNA molecule comprises nucleotide sequence as depicted in Figure 1.

33. The pGWYS1 DNA vector as depicted in Figure 4.

34. A recombinant *Pichia pastoris* cell containing the nucleic acid of claim 1.

20 35. A recombinant *Pichia pastoris* cell containing the DNA of claim 32.

36. A recombinant *Pichia pastoris* cell containing the DNA of claim 33.

25 37. A process for producing a chimeric protein comprising growing a recombinant *Pichia pastoris* cell containing the nucleic acid of claim 1 such that the encoded chimeric protein is expressed and secreted by the cell, and recovering the expressed and secreted chimeric protein.

30 38. A process for producing a chimeric protein comprising growing a recombinant *Pichia pastoris* cell containing the DNA of claim 32 such that the encoded chimeric protein is expressed and secreted by the cell, and recovering the expressed and secreted chimeric protein.

39. A process for producing a chimeric protein comprising growing a recombinant *Pichia pastoris* cell containing the DNA of claim 33 such that the encoded chimeric protein is expressed and secreted by the cell, and recovering the expressed and secreted chimeric protein.

5 40. The process of claim 37, wherein the expressed and secreted chimeric protein is recovered by a means comprising ion-exchange chromatography.

 41. The process of claim 40, wherein the ion-exchange chromatography being used is CM-Sephadex column chromatography.

10 42. The process of claim 40, wherein the ion-exchange chromatography being used is DEAE-Sephadex column chromatography.

 43. The product of the process of claim 37.

 44. The product of the process of claim 38.

 45. The product of the process of claim 39.

15 46. The product of the process of claim 40.

 47. The product of the process of claim 41.

 48. The product of the process of claim 42.

 49. A chimeric protein, said chimeric protein comprises, from N-terminus to C-terminus:

20 a) a first peptidyl fragment consisting of an amino acid sequence that has at least 40% identity to residues 1-50 of the B chain of native monellin, in which the percentage identity is determined over an amino acid sequence of identical size to the B chain of native monellin;

 b) a peptidyl bond, or a second peptidyl fragment consisting of 1-12 amino
25 acids; and

 c) a third peptidyl fragment consisting of an amino acid sequence that has at least 40% identity to residues 1-45 of the A chain of native monellin, in which the percentage identity is determined over an amino acid sequence of identical size to the A chain of native monellin,

30 wherein said chimeric protein is stable and a given amount of said chimeric protein is at least 100-fold sweet as compared to the identical amount of sucrose, and within said nucleic acid, codons which are preferably used by yeast cells are used.